Electroencephalographic changes of REM sleep in chronically sleep restricted mice

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Electroencephalographic changes of REM sleep in chronically sleep restricted mice

Directed by Professor Jeong-Hoon Kim and Jee Hyun Choi

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

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December 2014

This certifies that the Master's Thesis

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December 2014

Acknowledgements

Most of all, I appreciate my adviser, Dr. Jee Hyun Choi for both full support and motivation for advancing my research, and always encourage me to express my own opinion even when I lack of knowledge. Also I had chance to change my reluctant characteristic through her support. Besides giving me chance to acquire research skills, she always concerned about developing independent thinking and inference based on fact. Furthermore, through the study I could also think about role of scientist myself and responsibility for society.

I also express sincere thanks to Dr. Jeong-Hoon Kim. He also encourages me to express myself, and prosper my life to study science itself. His influence on me made me decide to peruse pure scientific motivation for my life time. I also thank my dissertation committee, Dr. Ho-Sung Jung for his deepest consideration and counsel.

Prior to thanking others, I would especially thank to one of my lab member, Dr. Eunjin Hwang for her responsiveness and consideration. She is not only my academic but personal role model. I also thank my Jee lab members and want to keep in close contact with Dr.Chungki Lee, Dr. Dongmyeong Lee, Soohyun Lee, Younginha Jung, and Beongjae Kwon, members in Center for Neuroscience, KIST for their friendship, encouragement and advice.

Last but not least, I also thank my loving family and my fiancé for their warm support.

Bowon Kim

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Abstract

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Chronic sleep restriction (CSR) has been shown to impair people's health and cognitive functions, as well as change sleep homeostasis. Previous studies have shown that CSR may impair non-rapid eye movement (NREM) sleep generation in addition to reducing NREM delta power. However, little is known about how CSR alters rapid eye movement (REM) sleep or EEG power spectra during REM. Here, we used a high-density EEG method in freely behaving mice to assess the REM sleep response to CSR.

Mice (N=9) were sleep deprived daily for 18-h using periodically rotating wheels, followed by 6-h of sleep opportunity that started at the beginning of each light period. This sleep restriction (SR) protocol was repeated for 5 consecutive days. High-density EEG was analyzed for only 2-h of each 6-h sleep opportunity (1-3h after the initiation of sleep opportunity), whereas conventional EEG data using skull screw electrodes was analyzed for the full 24-h experimental days.

The REM sleep time during the daily 6-h sleep opportunities increased as the SR days progressed, compared to the corresponding baseline levels. A power spectral analysis of the high-density EEG revealed that low theta power (5-7 Hz) increased significantly in the frontal cortex on SR day 1, then continuously decreased on SR3 and SR5; high theta power (7-10 Hz) was persistently elevated throughout all SR days, especially in the centro-parietal cortex. A close examination of theta oscillation revealed a

transition from unimodal to bimodal oscillation showing that a peak frequency at 7 Hz during baseline was split into two peak frequencies at 7 and 9 Hz.

Regarding REM gamma power, a gradual but significant increase in low gamma power (30-50 Hz) was observed near the prefrontal cortex especially on SR3 and SR5, while robust increases in high gamma power (70-100 Hz) were observed most significantly in the centro-parietal cortex on SR3. Additionally, the analysis of the cross-frequency coupling between theta phase and gamma power showed that modulation of theta on gamma oscillation was not altered during CSR.

Thus, CSR produces opposite effects on the low and high theta power of REM sleep in mice. In addition, this study indicates that CSR significantly increases REM gamma power while 18-h of acute sleep deprivation does not. Further studies are needed to determine if REM high theta power and gamma power actually correlates with the behavioral sleepiness and cognitive impairments observed in CSR.

Key words: REM, Gamma, Theta, sleep deprivation, CSR, CFC

ABBREVIATION

CSR = chronic sleep restriction

SR = sleep restriction

EEG = electroencephalography

REM = rapid eye movement

NREM = non- rapid eye movement

TWT = total wake time during sleep opportunity

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I. INTRODUCTION

1. Sleep

A. Sleep in general

Sleep is general phenomenon of nature, and evolutionary well conserved state of organism across species from invertebrate to all vertebrates. It is defined by the following; 1) reversible state regulated by circadian schedule and duration of wake period, 2) reduced responsiveness to external stimuli, 3) loss of consciousness[1].

Sleep consists of two distinct stage – namely non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. After transition from wake to NREM sleep, NREM and REM sleep occurs alternatively during human sleep. The transition of states (wake, NREM sleep, REM sleep) involved changes of neurotransmitter level. In addition, electroencephalogram (EEG) and network activity of brain was also changed in different states[2,3] (for review, McCarley, 2007; Viyazovskiy, 2014). Sleep regulation was well explained by 2-process-model. The model suggests that sleep/wake is regulated by homeostatic process which is amount of time spent awake and circadian process[4,5]. Accordingly, Sleep intensity and duration increased after prolonged wake state and deprivation of sleep. The increased intensity of sleep was suggested to correlate with slow wave activity (EEG power of delta band; 0.5 - 4 Hz) during NREM sleep[6-8]. The slow wave is most prominent EEG marker of sleep, reflecting synchronized firing and

cessation pattern of populations of neurons in cortex and thalamus[9,10]. Compared to NREM sleep, REM sleep response to sleep deprivation was not peculiar and less consistent. A study was reported that alpha band activity during REM sleep inversely represents to REM sleep pressure[11].

B. Rapid eye movement (REM) sleep

The terminology of REM sleep is defined by Aserinksky and Kleiman as they first found that rapid eye movements occurred regularly during sleep in human[12]. Another terminology of REM sleep is paradoxical sleep named by french researcher Jouvet in 1960s' because of its wake-like electroencephalogram pattern (EEG) with muscle atonia[13]. Beside of them, REM sleep has distinctive properties from NREM sleep or wake, which include: 1) desynchronized EEG pattern and theta oscillation in hippocampus; 2) postural muscle atonia; 3) rapid eye movements; 4) ponto-geniculo-occipital (PGO) wave; 5) myoclonic twitches; 6) temperature increase; 7) reporting dream in human; and 8) pronounced cardiorespiratory fluctuations[14,15].

Theta oscillation could be divided as two forms of theta. Type1 theta is low frequency theta (4 – 7 Hz) which was blocked by muscarinic antagonist. The other is type2 theta in high frequency (7 – 12 Hz) and was abolished by urethane anesthesia. Theta oscillation during REM sleep reveals both type of theta frequency. Mostly, slow frequency theta occurs during tonic REM sleep, having type1 theta property of muscarinic dependency. High frequency theta similar to type 2 intermittently showed during REM sleep[16-18].

C. Functional roles of REM sleep

A substantial body of evidence supports that REM sleep contributes to memory consolidation. Early and dogmatic works of REM sleep proposed a hypothesis that REM sleep facilitates memory consolidation as well as participates in offline information processing evidenced by studies showing that: (1) REM sleep deprivation studies have shown that REM sleep deprivation prior to or immediately after training impairs formation of a permanent memory and a sustained deprivation of REM sleep after learning interferes with memory consolidation[19], (2) REM sleep deprivation impairs memory acquisition as well as extinction[20], (3) a noticeable increase of *zif* expression,

which is a marker for long-term potentiation, was observed to be same level in REM sleeping rodents compared to the enriched-environment animals[21,22]. On the contrary, subsequent works of REM sleep has refuted this "REM sleep and memory " hypothesis with opposing evidences showing that (1) late NREM sleep deprivation rather than REM deprivation was most beneficial to memory[23], (2) an increased REM sleep duration was not correlated with learning ability in human and rats[24], (3) a suppression of REM sleep by selective administration of serotonin or norepinephrine re-uptake inhibitors after training enhanced rather than impaired the consolidation of skills or word-pairs in human[25]. However, further studies have shown that a more deliberated design of experimental paradigm might lead different outcomes in regards to role of REM sleep in memory consolidation. For example, a study with nap paradigm showed that the subjects with nap, who are expected to experience REM sleep during nap, showed a significant increase in emotional memory, whereas no changes in emotionally neutral memory, compared to the subjects who did not slept nap, indicating that REM sleep has role in consolidation of emotional human memories.

Besides of memory consolidation, REM sleep has shown to participate in multiple cognitive functions such as mood regulation, creativity, consciousness, etc. In particular, the positive role of REM sleep in mood regulation has been widely supported by evidences showing that: (1) a pre-sleep depressive mood was ameliorated after successive REM sleeps[26], (2) subjects after REM sleep in nap paradigm showed a reduced emotional sensitivity to negative emotion such as anger and fear, on the other hand, an increased sensitivity to positive emotion such as happy[27]. Interestingly, a REM sleep deprivation paradigm can be used to treat severely depressed patients although the effect is reversible once the patients are allowed to sleep[28,29]. In addition to mood regulation, unmeasurable or not-easily-assessible cognitive functions have been proposed as functional correlates of REM sleep. A preeminent dream theorist, J. Allen Hobson built a theory of protoconsciousness via neuroimaging data. He suggested that REM sleep may constitute a secondary conscious state (protoconscious state), providing a brain state a preparation period to undertake the integrative functions of waking consciousness equivalent to a functionally useful virtual reality model of the world[30]. Furthermore, a creativity leap role has been suggested from the observation that REM sleep stimulates or helps creativity by enhancing creative problem solving[31]. Notwithstanding, REM sleep

has been shown to neither be absence of distinct function in persistence of life nor lead detrimental consequences[32]. Hereafter, I conclude that REM sleep would have significant and comprehensive roles in cognitive function of human; however none of these hypotheses has been solidified yet.

2. Chronic sleep restriction (CSR)

Chronic sleep restriction (CSR), a type of sleep deprivation That reduces sleep time rather than eliminating it, mimics prevalent sleep loss in modern society due to vocational demands, addictive behavior, disturbed circadian rhythm, and insomnia induced by stress or clinical disorders. Though CSR studies in human have shown impairment in people's health and cognitive function as daily loss of sleep is accumulated[33,34], electrophysiological changes following chronic loss of sleep are rarely known.

A. Human studies

Empirically but also scientifically, sufficient sleep is crucial for human to maintain health and cognitive abilities. Empirical reports of increased accident rates following sleep deprivation can easily be obtained from people with certain types of jobs such as residents, truck drivers, and night-shift workers, which only allows for limited hours of sleep[35]. Several scientific researches has also shown that deprived sleep can cause both health problems and neurobehavioral deficits, including lapses of attention, working memory deficits, depressed mood, and perseveration of thought[36]. In particular, lapses of attention are especially sensitive to sleep loss and presumably reflect micro sleep, which is a short sleep-like period detected in changes of EEG signals during the wake state.

In studies that assigned participants into different sleep doses (from 4 hours to 9 hours), the number of lapses of attention increased systemically throughout the days of sleep restriction in groups scheduled to sleep for less than 7 hours a day[36,37]. However, same hours of sleep restriction may have different effects on each individual because his/her normal sleep schedule varies from each other's, although the median is generally considered to be 7 – 8 hours of sleep. Moreover, epidemiological evidence which controlled for compound effects such as psychosocial and clinical factors, excess sleep, or

sleeping for more than 8 hours, can be hazardous[38,39]. These studies eventually led to a question on the effect of modulations in sleep durations, and therefore, to studies that investigated whether mild chronic sleep restriction for people who have plenty of time to sleep caused impaired sleep quality and cognitive function (table1). In case of mild sleep reduction, CSR studies in humans revealed that the duration of sleep can be reduced within limits, without any subjective problem[38,40]. However, some of these studies show inconsistent results. They can be explained by several different reasons besides individual variability; first, sleep restriction might have different effects on subjects depending on what developmental stage they are in. Adults may be more resilient to sleep deprivation than children[41-43]. Furthermore, as shown in studies that used diverse types of tasks, only certain types of tasks are able to measure changes in neurobehavioral functions following sleep restriction. Finally and most interestingly, the subjective rating on the effects of sleep deprivation is inconsistent with objective measures of the effects of sleep deprivation.

B. Animal study

Animal studies were more focused on physiological changes during CSR rather than cognitive function. Particularly, many of them investigate homeostatic regulation of sleep after sleep deprivation, represented by NREM delta power. From the reports of NREM delta power changes during CSR, it was inferred that reduced sleep duration could be adapted in rat. The delta power initially increased on sleep restriction day 1, then returned to baseline level on restriction day 5, showing homeostatic needs of sleep was adapted[44,45]. However, in the other condition of sleep deprivation, rats did not show the adapted response[46]. Therefore, like human studies, the adaptation seemed to be dependent on sleep restriction schedule and the methods of sleep deprivation. For example, sleep deprivation by disk of water (DOW) was used for inducing mania state in rat, not falling asleep[47].

Table 1. Human studies of sleep restriction

Author	subjects	baseline	restriction	results	Measure, etc
Sleep homeostasis during repeated sleep restriction and recovery : support from EEG dynamics					
Akerstedt, T 2009	Male=9 (age 23-28)	23:00-7:00	4hour/ 5day 3:00-7:00	Homeostatic SWS time & power	EEG & power analysis
The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation.					
Van Dongen 2003	Adult = 48	8 hour 23:30–07:30	4,6,8 hour / 14 day	Homeostatic - Performance Adaptation - Subjective sleepiness	EEG & power analysis
Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study					
Belenky, G 2003	Male=50 (age 24-62) Female=16 (age 24-55)		3,5,7,9 hour / 7 day	Homeostatic - Performance Adaptation - Subjective sleepiness	Daytime performance
Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness					
Brunner, D. P 1993	Male=9, university students		4hour / 4 day	Homeostatic	rapid changes in the low delta band of NREM sleep, and slower, longer-lasting changes in the high delta band as well as the theta and alpha range of NREM sleep, REM sleep, and wakefulness.
Cumulative effects of sleep restriction on daytime sleepiness					
Carskadon, M. A. 1981	10 young adult	10 hour time in bed	5hour / 7 day	Stage 2, REM decreased SWS unaffected	
Sleep: effects of a restricted regime					
Webb, W. B. 1965	Male=9 Student (mean age 17.6)	7.5 hour ~	3 hour / 8 day Pair 1) 23:00-2:00 2) 2:30-5:30	Stage 4(NREM) increased	EEG for stage scoring Before sleep performance test

Investigating changes during REM sleep during CSR could give the functionally important physiological alteration following chronic sleep deprivation. We are going to show electrophysiological changes during REM sleep along with chronic sleep loss as following, 1) Increased total REM sleep time and duration but decreased NREM and total sleep time in sleep structure, 2) changes of topographical power distribution, 3) power spectra revealed theta band broadening, 4) cross-frequency-coupling strength value was obtained.

For our best knowledge, this is the first study in aspect of CSR conducted in mice and Results focused during REM sleep through CSR. Mainly, we would report the electrophysiological changes during REM sleep along the chronic sleep losses are accumulated in this study.

II. MATERIALS AND METHODS

1. Animal preparation and surgery

A. Animals

Male C57BL/6 and 129S4/Svjae hybrid F1 mice at 12weeks were used for surgery (N=9), at 13weeks when EEG was recorded. They were hosed in groups of three to six while 3 to 12weeks of age, and individually separated after electrode surgery. Animals were maintained under 12-h light/12-h dark cycle (light on at 8:00a.m.) with freely accessible food and water in room of which temperature and humidity were controlled. All procedures were approved by the Institutional Animal Care and Use committee at Korea Institute of Science and Technology, following Act 1992 of the Korea Lab Animal Care Regulations and associated guidelines. All efforts were made to minimize animal suffering and to reduce the number of animals used.

B. Surgery

Animals were anesthetized by intraperitoneal injection with ketamine-xylazine cocktail (120 and 6 mg/kg) and 2 to 3 times of additional dosage (a third of the original dose) were given for approximately three hours of surgery. After confirmed anesthetized condition, mouse was positioned on a stereotaxic apparatus (David Kopf Instruments, model 902, Tujunga, CA, USA). After head barbered, Skull was exposed with scissors to positioned screw and high-density electrodes.The high-density microarray electrodes followed implanting procedures previously described in Lee et al, 2011. Briefly, the exposed skull wiped with saline and tap water soaked cotton balls. Then, a polyimide based 40 channel microarray (HD-EEG electrode) was placed on skull, aligned vertically with a line joining bregma point and lambda point(LP), of which 5th wing was fitted right above the BP and left to dry up .

For conventional screw EEG recording, 3 screw electrodes (Stainless tapping screw, 0.8*4.8mm, Nitto Seiko Co., Japan) were tightened into each holes (bar size 1/2HP, 0.6mm in diameter) drilled on exposed skull surface between wings of HD-EEG electrode; one on the frontal area (1.5mm anterior and -2mm lateral to BP), one on the parietal area (-2mm anterior and 4mm lateral to BP), and one on the interparietal bone

above cerebellum for both reference and ground electrode (-6mm anterior and -2mm lateral to BP). 2 to 3 additional screws were also implanted for supporting electrodes. All electrodes were secured to the skull with self-curing dental cement (Vertex-Dental, Zeist, Netherlands). After curing, skin of exposed skull area was sutured before maintained in an individual cage.

2. Chronic sleep deprivation

A. Habituation

Before went on experiment, Animals were individually moved into transparent cylindrical cage in electrically isolated box (size 50w*60l*60h (cm)).. In there, mice were habituated to different light-on time schedule (light on at 9:00a.m. or 1:00p.m.) with electric wire connected with electrodes on mouse head. Habituation days differed as amount of changes of time schedule for experiment from original time of mouse room. For example, habituation to 1:00p.m. experimental time schedule required at least 6 days before experiment. Then, last 2 days of habituation and each 2 hour a day, to automatically moving wheel with fixed time schedule for sleep deprivation . EEGs were recorded following at least a week to two weeks of recovery time including habituation

B. Experimental design

Chronic sleep deprivation protocol originally from Harvard medical school (Kim el al, 2012) was applied and modified for mice study. Experiment went on 9 days, which consist of baseline (BL, 1 day), sleep restriction (SR, 5 days) and recovery (R, 3 days). All experiments day was scheduled to be started 6 hour after the light onset (Zeitgeber time (ZT) 6). Therefore, Baseline recording was started ZT 6 for 24 hours and from ZT 6 on next day of baseline, 18 hour Sleep deprivation procedure was started, followed by Sleep opportunity (SO) for 6 hours (From ZT 0 to ZT 6). Then, Sleep deprivation and SO was repeated 5 times for 5 days of sleep restriction. After the end of 5-day-sleep restriction, mouse was not disturbed for 3 days of recovery under EEG recording. Conventional screw EEG was recorded continuously for all 9 days of experiment, briefly stopped recording for approximately 5-s in a day or two to save. Also, High-density EEG

was saved for ZT0-3(i.e. the first 3 hours of the SO) of BL, SR1, SR3, SR5, R1 and R3 day.

C. Sleep deprivation protocol

Sleep deprivation was performed with periodically rotating wheels (5.5 inch in diameter × 2.3 inch in width, Lafayette Instrument, Lafayette, IN, US, #80860) programmed on a repeated cycle of 4-s on (approximately 2.3 RPM) and 2-s off schedule during the daily 18-h periods of sleep deprivation. Mice had free access to food and water during sleep deprivation periods. After 18-h sleep deprivation, animals were transferred to their recording cages for the 6-h sleep opportunities.

3. Data analysis

A. EEG signal trace and power spectrum

Conventional screw EEG data were converted to ASCII format using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA, USA). SleepSign software (Kissei Comtec Co., Japan) was used to manually score 10-s EEG epochs of the conventional EEG data as wake, NREM sleep or REM sleep. Epochs containing EEG artifacts were excluded from the power spectral analysis. Depending on the particular analysis, wake, NREM sleep and REM sleep time, NREM episode duration and numbers as well as delta power, were determined in 2, 6, 18 or 24-h time blocks.

The HD-EEG data were further analyzed separately with MATLAB (Mathworks, Inc. Natick, MA, USA). The HD-EEG signals were divided into NREM epochs based on the synchronized data from the manual scoring of the conventional EEG and the motion sensor. FFT was applied to each NREM epoch of the HD-EEG and FFT's squared magnitudes between 1-4 Hz were averaged. The values of NREM delta power during ZT1-3 were averaged for each day in each mouse. Power ratio to baseline day for each sleep restriction and recovery day was computed as follows:

$$power \quad ratio = \frac{\langle P_{DAY} \rangle - \langle P_{BASE} \rangle}{\langle P_{BASE} \rangle},$$

where $\langle P_{BASE} \rangle$ and $\langle P_{DAY} \rangle$ are the averaged delta power of NREM epochs for baseline day and the day of comparison, respectively.

The channels with contact impedance higher than 1 M were excluded in the analysis. The topographical mapping was represented by a contour plot on the brain surface which were rendered by 'spm_surf' function in SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) using the mouse magnetic resonance microscopy atlas (downloadable in http://www.loni.ucla.edu/). The fictitious points for the contour were estimated with a cubic spline interpolation method on the imaginary 2-D 250 x 250 meshgrid based on the coordinates of microarray.

B. Phase-amplitude coupling

To quantify phase-amplitude cross-frequency coupling between two ranges of frequencies of interest, we employed the modulation index (MI) described in Tort et al. (Tort et al., Proc Natl Acad Sci USA (2008) 105:20517–20522). MI is based on a normalized entropy measure previously described in Hurtado et al. Briefly speaking, firstly, the raw signal is filtered by narrow band zero-phase bandpass filter and then the phase of the slower signal component is obtained by the Hilbert transform. Next, the phases of the slower signal are binned into 20° intervals, and the mean amplitude of faster signal component over each phase bin is calculated according to definition of Shannon entropy, $H = -\sum_{j=1}^{N} p_j \log p_j$, where p_j is determined by amplitude of faster signal component multiplied by phase of slower signal component at each phase bin. The MI is finally obtained by the definition, MI= ((H_max-H)) ⁄ H_max , where is H_max=log(number of phase bin) meaning uniform distribution of p_j. A zero MI value indicates a uniform distribution of p_j and means no phase dependence of faster component. The larger MI value indicates a stronger modulation of faster component by phase of slower component. The comodulogram plot is drawn to represent the scale of MI values between phase of slower component and amplitude of faster component by a Matlab code developed by Tort et al. (Tort et al., J Neurophysiol (2010) 104:1195–1210).

C. Statistical Analysis

For statistical comparisons of sleep-wake parameters across BL, SR1-SR5 and R1-R3 conditions, a repeated-measures analysis of variance (ANOVA) test was used. Post-hoc comparisons, when indicated, were made using the Fisher's LSD test. For comparing 3 days of recovery sleep from the baseline sleep in 2-h intervals, paired two-tailed t-test was used. Comparisons were considered significant if $p < 0.05$. All error bars in the figures and the range following the mean value in the text represent standard error of mean.

III. RESULTS

1. Effect of chronic sleep restriction on sleep structure

A. Chronic sleep deprivation reduces the total sleep, but enhances the REM sleep

The sleep structures of 6 hours of sleep after 18 hours of sleep deprivation were analyzed. During SR days, a progress decrease in total sleep time was observed (Fig. R1-A). In acute sleep deprivation (SR day 1), the total sleep time was not statistically different compared to baseline sleep (paired t-test, $p=0.380$). On the other hand, the total sleep time decreased statistically significantly in chronic sleep deprivation period (paired t-test, $p=0.006$ for SR day 3 and $p=0.001$ for SR day 5). Quantitatively, $16 \pm 13\%$ and $27 \pm 14\%$ of total sleep time decreased for SR day 3 and SR day 5, respectively, which correspond to 7.0 ± 5.6 min and 11.1 ± 6.4 min of sleep loss per hour. This reduction in total sleep time was fully recovered after two days of recovery sleep (paired t-test, $p=0.051$ for R day 1 and $p=0.804$ for R day 3). Decomposition of NREM and REM sleep showed that the reduction of total sleep time during chronic sleep deprivation was contributed mainly by reduction in NREM sleep time (Fig. R1-B). Interestingly, REM sleep time increased statistically significantly during sleep deprivation period (paired t-test, $p=0.0002$ for SR day 1, $p=0.0017$ for SR day 3, and $p=0.0005$ for SR day5). During baseline, mice slept REM sleep for 3.8 ± 1.2 min per hour, and this hourly REM sleep time increased by 2.2 ± 1.0 min, 1.8 ± 1.2 min, and 2.3 ± 1.2 min for SR day 1, 3, and 5, respectively. This increase in REM sleep time returned to the baseline level in the first recovery day (paired t-test, $p=0.632$), but increased again by 0.9 ± 1.1 min in the third recovery day (paired t-test, $p=0.032$). The relative distribution of NREM and REM sleep time shows that the ratio of REM sleep to NREM sleep increased during chronic sleep deprivation (Fig. R1-C).

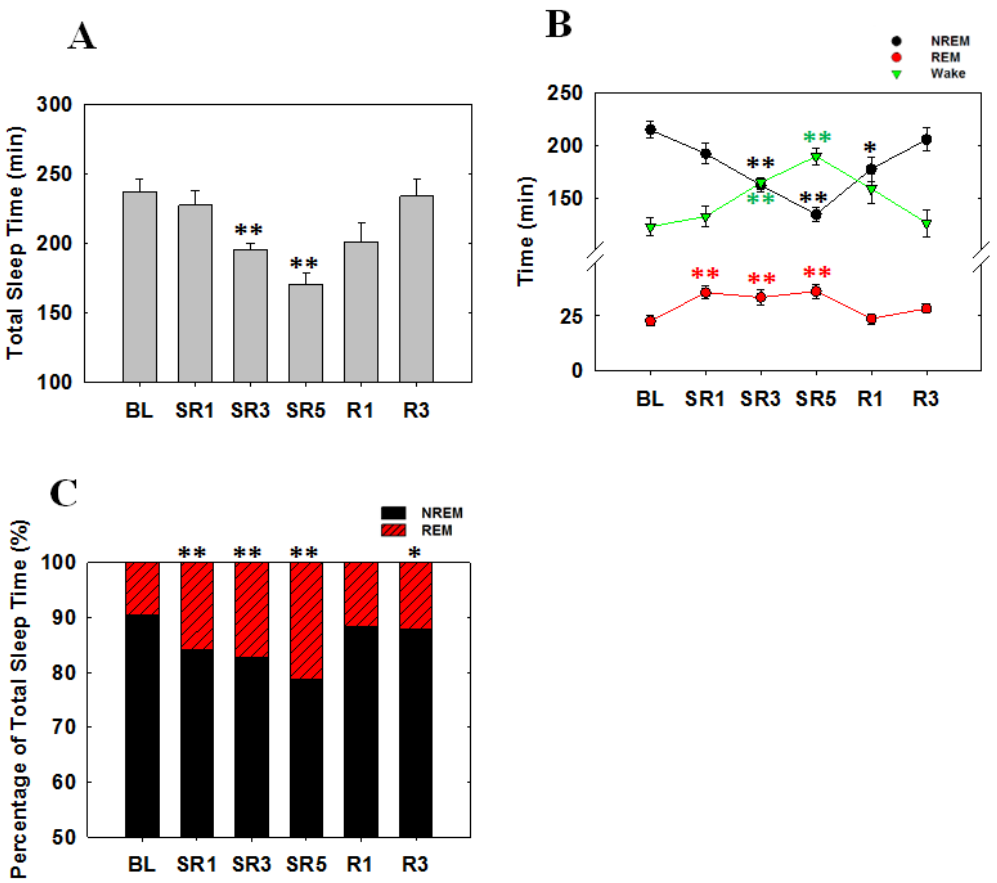

Figure 1. Effect of chronic sleep deprivation on sleep times.
(A) Total sleep time during 6 hours of sleep opportunity following 18 hours of sleep restriction, corresponding to an addition of NREM and REM sleep periods (N=9) . A statistically significant reduction in total sleep time was observed in SR3 and SR5. The errorbars indicate a standard error mean. (B) Average of NREM (black), REM (red), and wake periods (green) (N=9). Sleep deprivation influences different vigilance states in a distinctive way. Compared to natural sleep, sleep deprivation leads a significant reduction in NREM sleep period in a monotonic way, whereas elevates REM periods with a similar level. The errorbars indicate a standard error mean.(C) The relative portion of NREM (black area) and REM sleep (red area) time out of total sleep time plotted in percentage. A significant increase of ratio of REM to NREM sleep in terms of time is observed. BL = baseline day (natural sleep); SR1 = 1st day of sleep restriction; SR3 = 3rd day of sleep restriction; SR5 = 5th day of sleep restriction; R1 = 1st recovery day; R3 = 3rd recovery day. Asterisks (**) indicate P-value lower than 0.01 and P-value ranging 0.01 to 0.05 is shown by (*)

B. Chronic sleep deprivation lengthens REM sleep episodes

The mean lengths of NREM and REM sleep episodes were calculated to investigate the features of NREM and REM sleep. The episode length of REM sleep progressively increased during SR days followed by returning to baseline level in recovery sleep, however, the episode length of NREM sleep maintained to the baseline level (Fig. R2-A). The mean duration of individual REM sleep was 77 ± 10 sec in baseline sleep, and during chronic sleep deprivation, this value increased statistically significantly by 24 ± 9 sec, 26 ± 11 sec, and 21 ± 22 sec in SR day 1, 3, and 5, respectively (paired t-test, p=0.0001 for SR day 1, p=0.0001 for SR day 3, and 0.02 for SR day 5 compared to baseline sleep). Figure R2-B depicts the ranges of episode length of REM sleep as well as their occurrence marked by the size of the marker in individual mice. It is observed that longer REM sleep episode occurred more prevalently as the sleep deprivation continues.
In NREM sleep, a statistically significant increase in NREM episode length was found in SR day 1 (paired t-test, p=0.001 by 64 ± 38 sec) and SR day 3 (paired t-test, p=0.023 by 37 ± 40 sec), implying sleep deprivation leads deep sleep longer. However, this longer deep sleep returns to the baseline as the sleep deprivation sustained (paired t-test, p =0.3 for SR day 5).

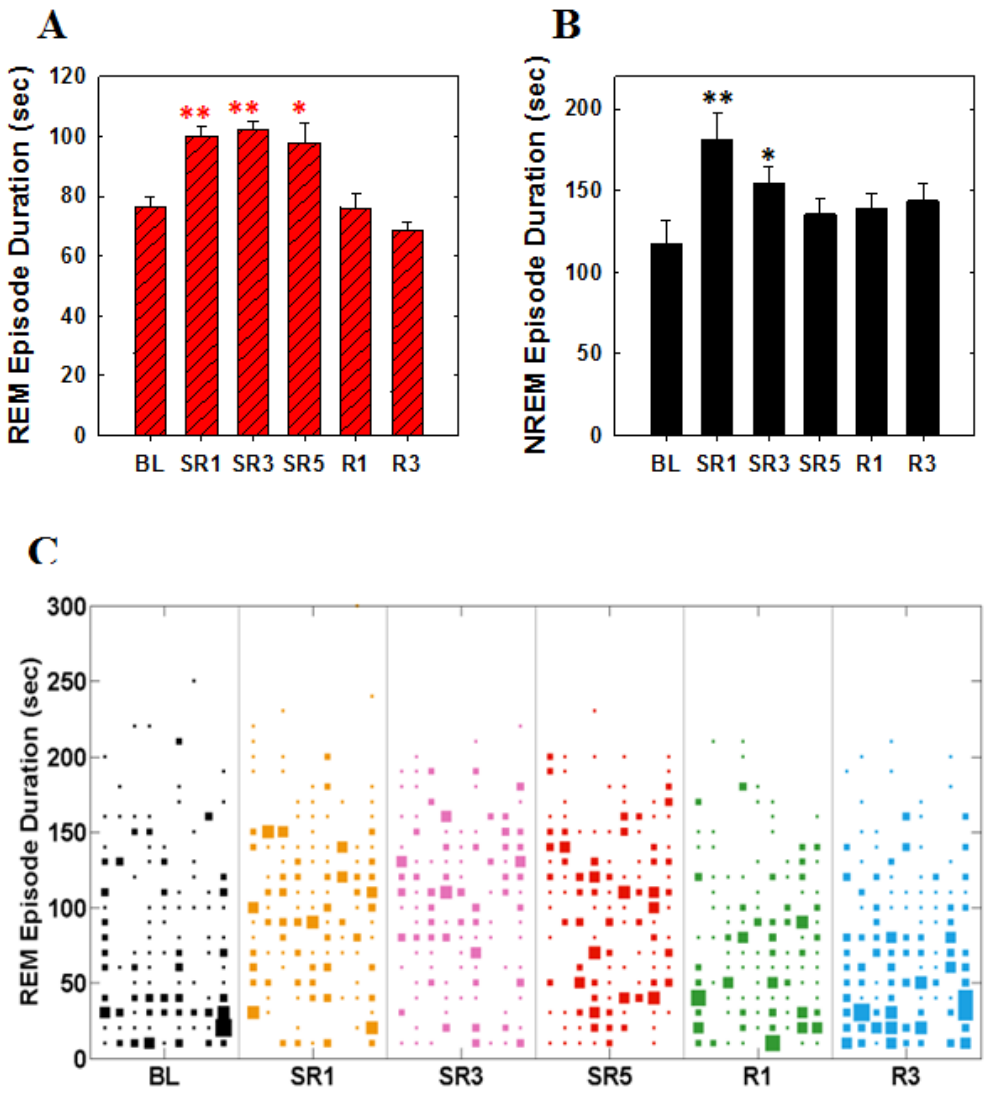

Figure 2. Effect of chronic sleep deprivation on sleep stages.

(A) Mean length of REM sleep episode (left panel) and NREM sleep episode (right panel) averaged for 9 mice. Each value was calculated by dividing total time of REM or NREM sleep time by number of their occurrence. During sleep deprivation, individual REM sleep stage was longer compared to natural sleep. However, NREM sleep stage was influenced only in the first day of sleep deprivation in an increasing pattern. Asterisks (*) indicate P-value lower than 0.01 and P-value ranging 0.01 to 0.05 is shown by value (paired t-test). (B) Scatter plot of REM episode length plotted for individual mouse and days. Each column corresponds to individual mouse. The size of square is proportional to occurrence rate of same length detected. During SR, each REM episode was effectively longer compared to natural sleep.

C. NREM sleep transit to REM sleep more frequently in acute sleep deprivation

Figure R3-A summarizes the influence of chronic sleep deprivation on transition rates between different sleep stages. Most noticeably, sleep deprivation significantly reduced the arousal frequency significantly but mostly from the NREM sleep stage. The animals waked up approximately 9 times less every hour during SR days and R day 1 (9.0 ± 5.4 less compared to baseline, averaged from SR1 to R1 days. No statistical difference between days). Since the average waking frequency is approximately 20 times per hour, the animals waked up 50% less in high sleep pressure. The calculation of transition rate from REM sleep to wake stages showed that the animals waked up less mostly from deep sleep. Accordingly, the transition from NREM sleep to REM sleep increased significantly but only in the first sleep deprivation day (paired t-test, $p=0.032$ for SR day 1, $p=0.583$ for SR day 3, $p=0.134$ for SR day 5). Figure R3-B is a schematic diagram for the SR effects on the transition of the sleep-wake cycle. The transition between wake-NREM sleep stages was inhibited during sleep deprivation days (SR day 1-5), whereas the transition from NREM to REM sleeps was promoted in the acute sleep deprivation condition (SR day 1).

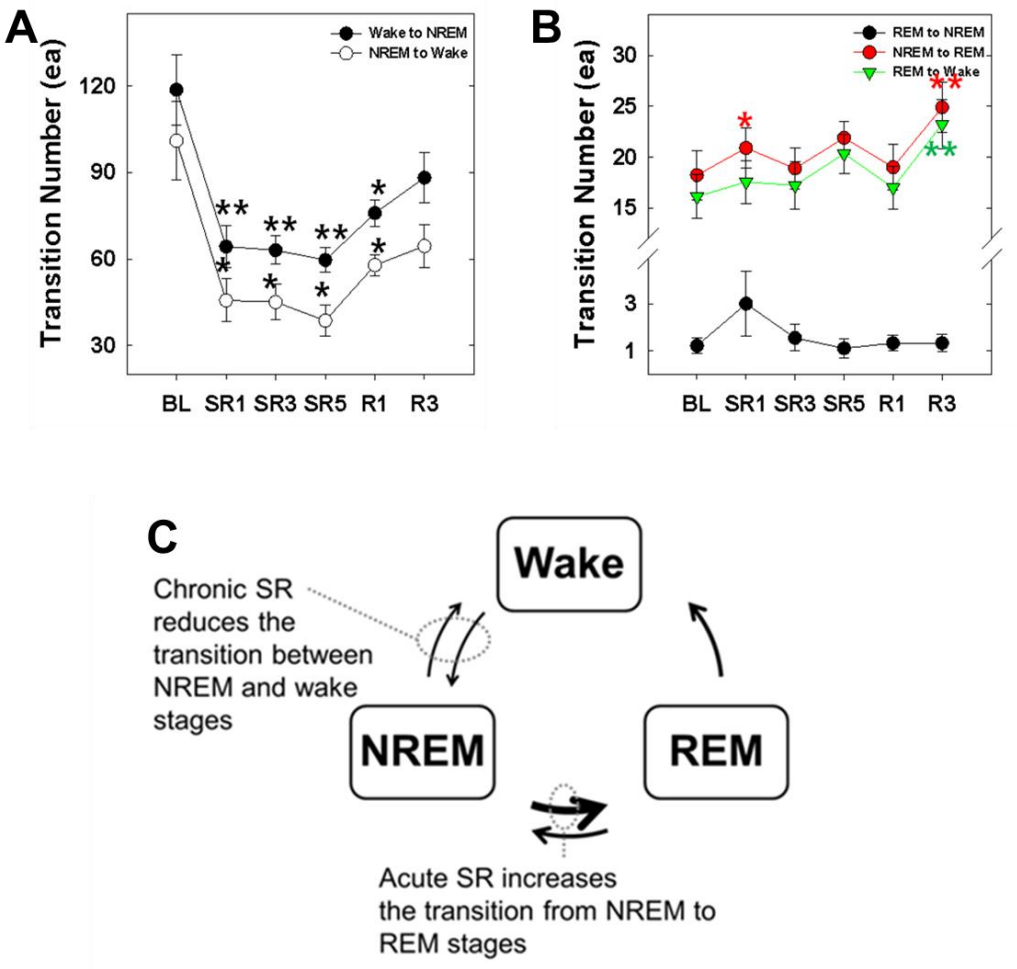

Figure 3. Effect of sleep deprivation on sleep stage transitions.
(A) The average number of transition from or to REM stages during 6 hour of sleep opportunity (N=9). Transitions from NREM to REM stages (red) and from REM to wake stages (green) were not influenced by sleep deprivation. Transition from REM to NREM (black) increased in SR1, but below the statistically significant level ($p>0.05$). (B) Average number of transition between NREM and REM stages. Significant drops were observed both in NREM to wake transition (white) and wake to NREM transition (black) throughout sleep deprived days. (C) A schematic diagram showing the influence of sleep deprivation on the transition of the sleep-wake cycle. The mutual transitions between NREM sleep and wake stages decreased during chronic sleep restriction, represented as thinner arrows. The transition from NREM to REM sleep stages increased only in the first day of sleep restriction, represented as thicker arrows, whereas the transition from REM to NREM or Wake remained to be unchanged.

2. Sleep deprivation alters the theta and gamma oscillations during REM sleep

We draw the topographies at each frequency to divide the frequency range according to the level of being influenced by acute and chronic sleep deprivation (Fig. R4). 1, 2, and 3 Hz frequency bins were used for delta and theta, beta and low gamma, and high gamma oscillations, respectively. In SR day 1, overall increase of power was monitored in all the frequency bands. Particularly, theta oscillation in the frequency range from 8 to 12 Hz increased significantly in centro-parietal cortex, and in the same region, fast gamma in 90 – 100 Hz strongly increased. Note that predominance for increased power was observed in the central parietal cortex. In SR day 5, most noticeably two-mode oscillation was observed in theta frequency band. In centro-parietal cortex, low theta power (6 – 7 Hz) decreased significantly compared to baseline REM sleep, whereas high theta power (8 – 12 Hz) increased significantly compared to both baseline and SR day 1. Also, a predominant increase of beta oscillation (17 – 35 Hz) was observed in the somatosensory-motor cortex and a noticeable increase of 40 Hz gamma oscillation in anterior frontal cortex was observed. In SR day 5, the fast gamma in the centro-parietal cortex increased more strongly as well compared to SR day 1.

Figure R5 summarized the topographical changes of theta and gamma oscillation in REM sleep during sleep deprivation and recovery sleep compared to baseline sleep averaged across all mice. The opposite behavior of low and high theta oscillation during chronic sleep deprivation emphasizes the biphasic response of theta oscillation to sleep pressure. The color represents the relative power change (i.e., $(P_{SD}-P_B)/P_B$ or $(P_{SD}-P_B)/P_B$). We divided the theta oscillation into low theta (5 – 7 Hz) and high theta (7 – 10 Hz) bands and the gamma oscillation into low (30 – 50 Hz) and high (70 – 100 Hz) bands. The gray-color map blow the topography shows the cortical regions affected by sleep deprivation. The white and black regions represent the regions of increased and decreased EEG power compared to baseline, respectively. Low theta power in centro-parietal cortex decreased significantly in chronic sleep deprivation condition; whereas increased region of high theta power reduced as sleep deprivation continues. In case of gamma oscillation, low gamma power increased in anterior frontal cortex during chronic sleep deprivation, whereas high gamma power increased in centro-parietal cortex during chronic sleep deprivation.

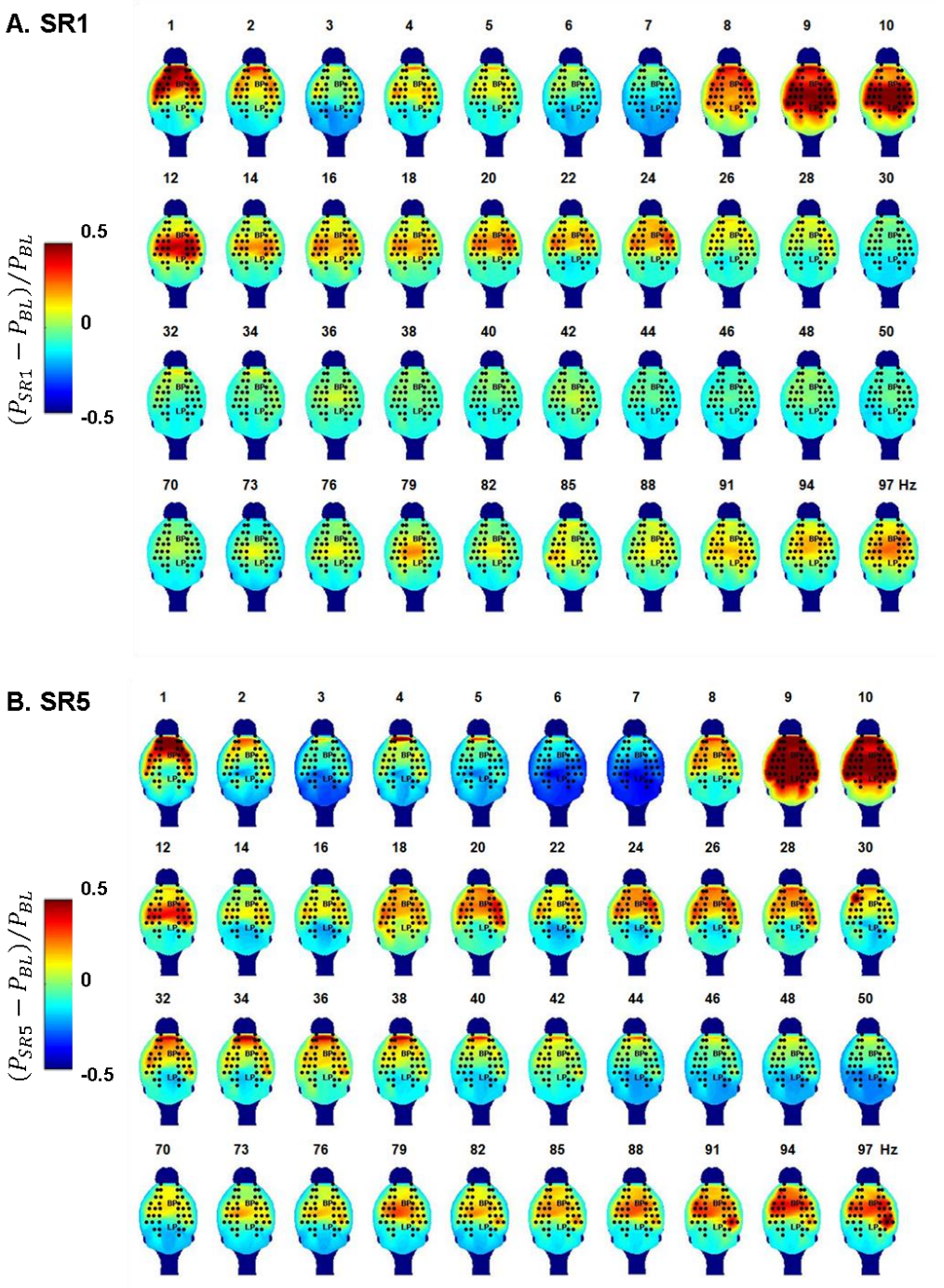

Figure 4. Topographical changes of spectral power of EEG in acutely (SR1) and chronically (SR5) sleep deprived condition.

The group average of relative power changes compared to normal sleep, $(power_{SR}(f) - power_{BL}(f))/power_{BL}(f)$ are depicted at each frequency (N=9). The top and bottom of topography correspond to anterior and posterior sides of mouse brain, and the electrode positions are marked as black dots. The number represents the

frequency of observation. 1, 2, and 3 Hz bin were used for delta and theta, beta and low gamma, and high gamma oscillations, respectively.

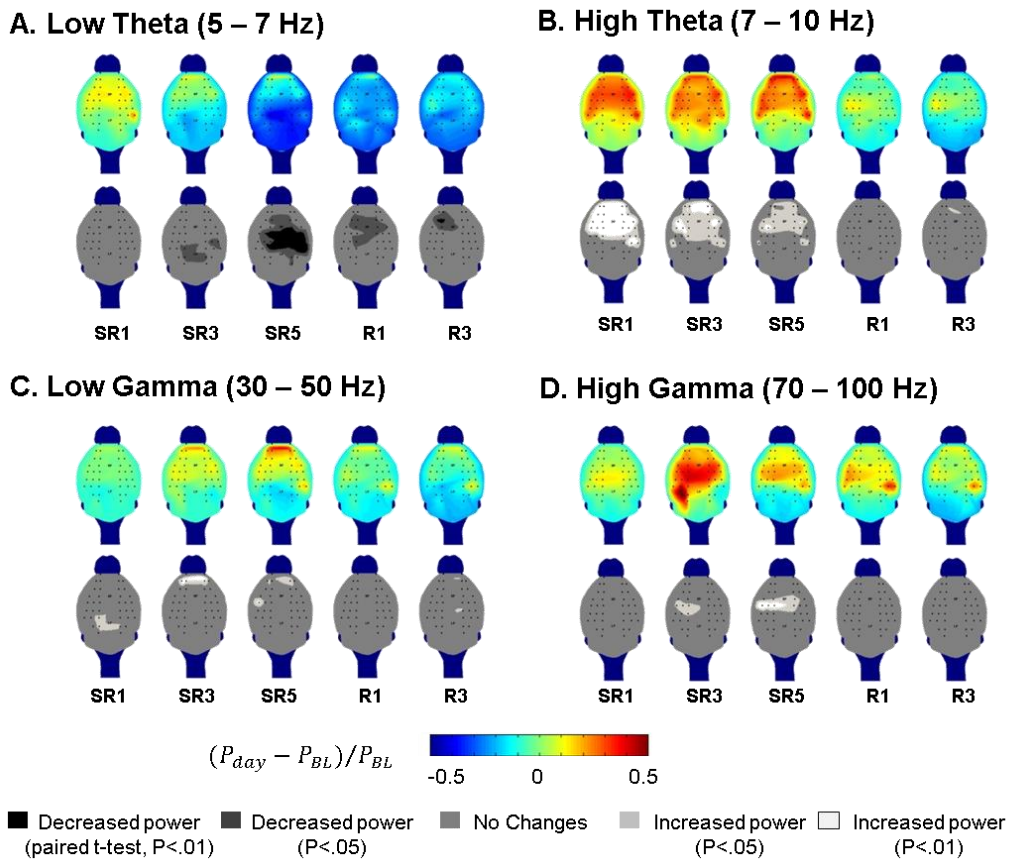

Figure 5. The topographical changes of theta and gamma powers in REM sleep during sleep deprivation. The color graphs in upper row are the topography of relative power, and the grey graphs in lower row show the statistically significantly different regions. The group average of relative power was obtained (N=9). We used the same definition of relative power in Fig. 4. The topographies for (A) low theta (5 – 7 Hz), (B) high theta (7 – 10 Hz), (C) low gamma (30 – 50 Hz), and (D) high gamma (70 – 100 Hz) are shown. It is monitored that the behaviors of low theta and high theta are opposite during sleep deprivation. Both low gamma and high gamma powers increased, but in different cortical region, where low gamma increased mostly in anterior frontal cortex and high gamma increased in centro-parietal cortex.

3. Chronic sleep deprivation perturbs REM theta oscillation

The biphasic behavior of theta oscillation may be due to frequency shift of theta oscillation or transition of theta oscillation from unimodal to bimodal oscillation or mixture of both. To find the origin of this biphasic response, the peak frequency and full half at half maximum (FHHM) of theta power were calculated from the averaged power spectrogram of parietal EEG averaged over 9 mice (Figure R6-A). Figure R6-B shows the frequency bifurcation as sleep deprivation continues. Here, the peak values of the frequencies within theta frequency bands were picked up from the zero second derivatives of the power spectrum of Fig. R6-A. During baseline sleep, the number of dominant frequencies is confined to lower frequencies near 7 Hz, and is clearly seen that from SR day 1, the higher frequency is observed and become prevalent in SR day 5. More close investigation showed that 5 out of 9 mice showed a blue-shift in the spectrum and 3 out of 9 mice transit from unimodal to bimodal oscillations by presenting two peaks in the spectrum. 1 mouse did not show any change in the peak frequency of REM theta oscillation. The broadening of theta oscillation was also observed in the full-width at half maximum (FWHM) plotted with respect to the day (Fig. R6-C). A statistically significant broadening of theta oscillation was observed in chronic sleep deprivation (SR day 3 and 5) and recovery sleep (R day 1 and 3). The theta power at peak frequency did not show any dependency of sleep deprivation (data not shown).

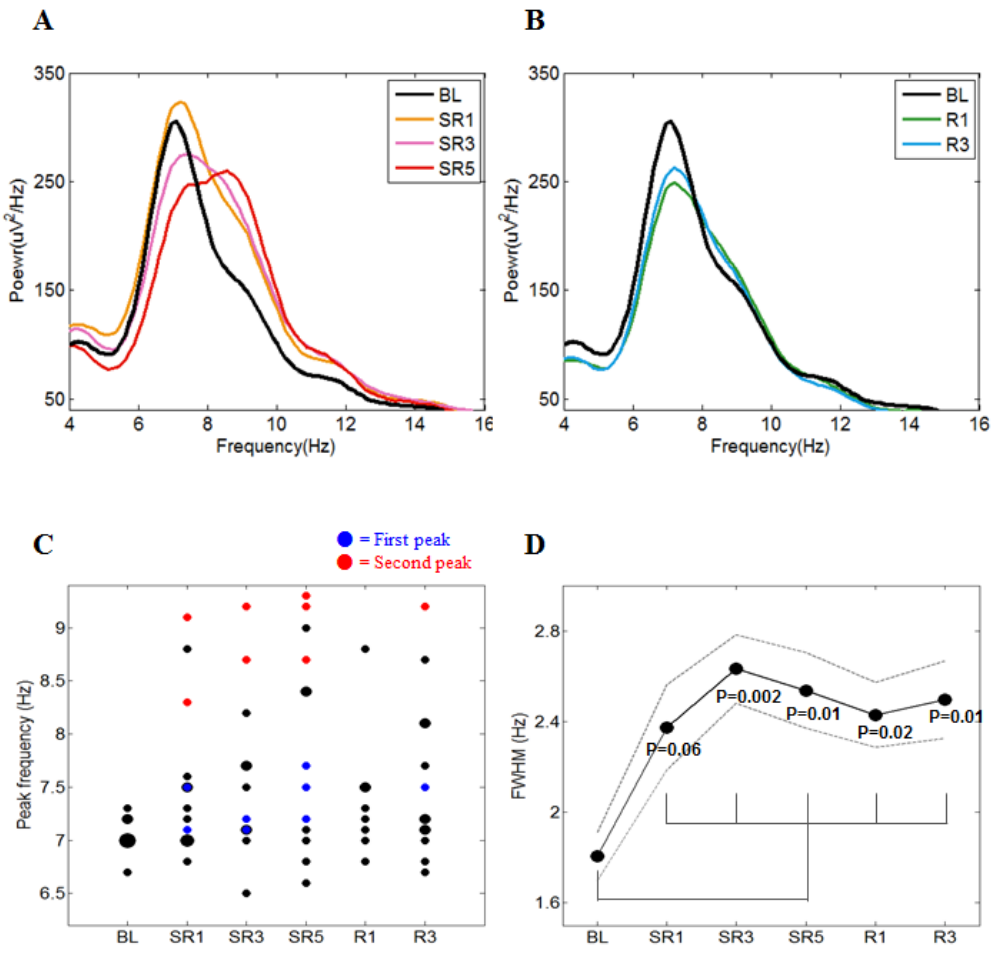

Figure 6. The effect of sleep deprivation on theta oscillation during REM sleep.
(A, B) Averaged power spectrogram of parietal EEG (N=9). Baseline = black, SR1 = orange, SR3 = pink, SR5 = red, R1 = green and R3 = blue line. The spectra show that peak of theta oscillations (5 – 10 Hz) tend to broaden and in SR5, double peaks were observed. (C) Frequency bifurcation diagram. The peak frequency was manually detected from the power spectrum. In case of the double peaks in the theta range, for example, spectrum on SR5 in (A), first peak is marked as blue dots, and second peak dotted by red color. A bifurcation of peak frequency throughout the sleep deprivation period is monitored. (D) Full width at half maximum (FWHM) of theta oscillations. FWHM was determined as the difference between the two frequency values at which their power is equal to half of the peak power in the power spectrum in theta band. The spectral width of theta oscillation increased significantly during chronic sleep deprivation, and did not return to the baseline value in the recovery sleep

4. Cross-frequency theta-phase gamma-amplitude coupling during chronic sleep deprivation

Recently, Scheffzuk et al found that coupling between theta phase and gamma amplitude was more stronger during REM sleep as compared with active wakefulness (PLoS One 2013), suggesting a state-dependent modulation of gamma oscillation by theta phase. A visual inspection of EEG traces from centro-parietal cortex already showed that gamma amplitudes were modulated by theta phase.

To search the brain region and frequency bands existing the theta-gamma coupling, we first analyzed the EEG during normal REM sleep by applying a comodulation method as described elsewhere (Tort et al., PNAS, 2008). As shown in Fig. 7B, the comodulograms reveal prominent theta-gamma coupling in centro-parietal cortex and substantial modulations were observed in high gamma (70 – 100 Hz) and very fast oscillation (120 – 150 Hz) bands by theta phase (7 – 10 Hz). Particularly, local maximal modulation were found above medial parietal association cortex (approximately, AP:-2 mm, ML:±1 mm, marked as A in Fig. 7B) at 7 Hz phase and 70 Hz amplitude, and above lateral parietal association cortex (approximately, AP:-2 mm, ML:±1 mm, marked as B in Fig. 7B) at 7 Hz phase and 150 Hz amplitude. The averaged amplitudes of 50, 70, and 150 Hz oscillations in these two regions were plotted with respect to 8 Hz theta phase. The phase-power plots revealed negative value of theta phase for peak amplitude. The peak phase was converted to delay in msec, and 50, 70, 150 Hz oscillations showed maximal amplitude with 1.5, 1.6, 0.4 msec delay with respect to zero phase of theta.

We tested if the peak amplitude and delay are influenced by sleep deprivation. Non-parametric statistical tests showed that the peak amplitude is not influenced by sleep deprivation ($p>0.05$, Kruskal-Wallis test), whereas the delay values in SR1 and SR3 were reduced statistically significantly ($p<0.05$, Kruskal-Wallis test). As compared in differential comodulation plots, a significant increase of theta phase and high gamma amplitude was observed particularly in 8 – 9 Hz phase and 70-90 Hz amplitude in SR1. As sleep deprivation continues, the comodulation plot shows a biphasic behavior of theta: with respect to high theta phase, the significant increase of modulation on high gamma amplitude was sustained or slightly increased. On the other hand, a significant decrease of low theta phase and gamma modulation was monitored in both SR3 and SR5 (Fig. 7C). A

correlational study showed any positive or negative correlation of theta-gamma modulation indices to sleep deprivation days (data not shown).

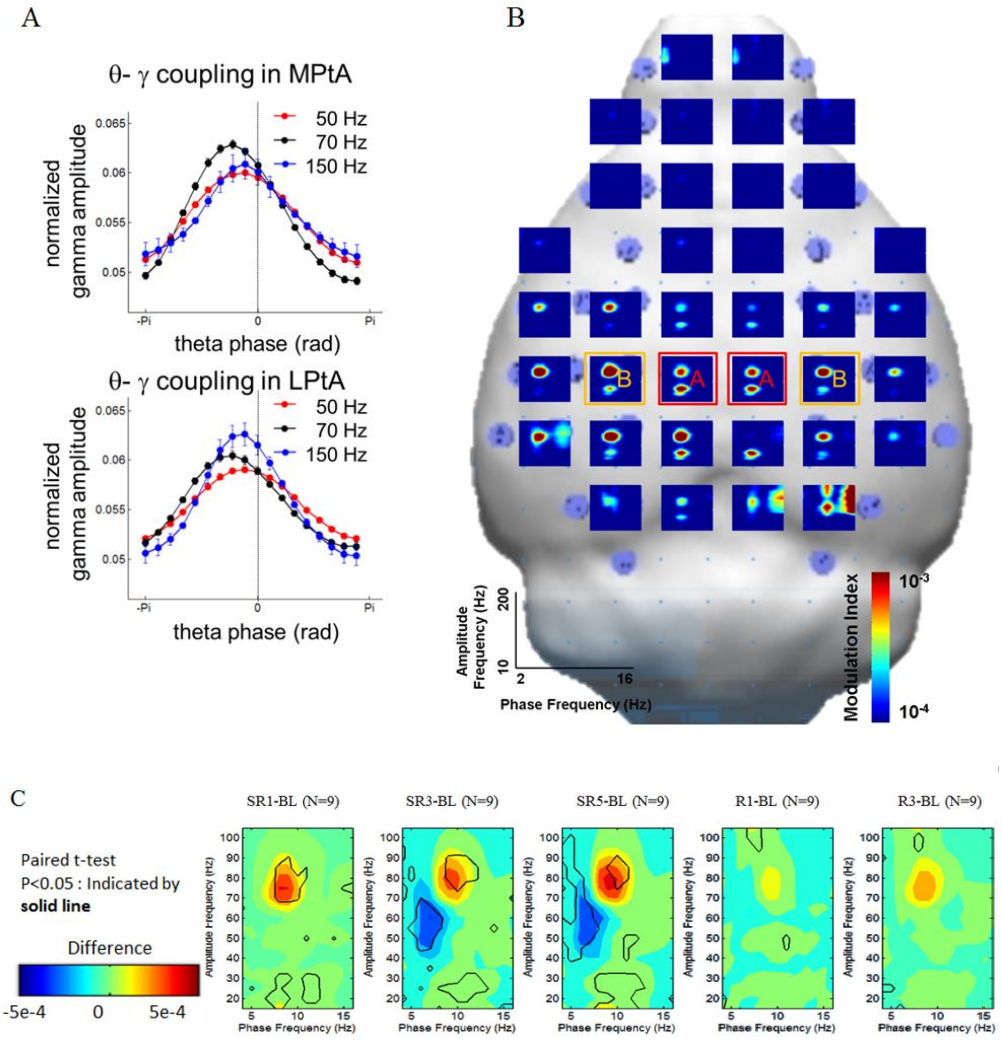

Figure 7. The theta phase modulation of gamma amplitude during CSR.
(A) Phase-amplitude comodulogram computed for REM sleep in natural sleep recorded at different cortical locations. Strong phase coupling of high gamma oscillation around 70 Hz and very fast oscillation around 150 Hz are observed in medial parietal association cortex (MtPA) and medial parietal association cortex (LtPA), respectively. (B) Mean amplitudes per theta phase for theta period were defined for low gamma (50 Hz; red), high gamma (70 Hz; black), and very fast oscillation (150 Hz; blue) in MPtA and LPtA. (C) Differential comodulogram computed as comodulation in test day minus baseline day values. Decreased strength indicated by blue, increased strength by red color. The solid line defined the area of significantly different values, ($p<0.05$, paired t-test, N=9). During chronic sleep deprivation, high gamma oscillations are more modulated by high theta phase, whereas less modulated by low theta phase.

IV. DISCUSSION

1. EEG power maps: response to sleep pressure

We found that percentage of REM sleep increased while total sleep time decreased through CSR. During REM sleep electrophysiological power in each frequency calculated by 1-Hz bin is overall increased during CSR, having distinct brain region of main increase. Only lower theta frequency revealed decreased power compared to baseline. In close looking for spectroanalysis from averaged parietal signal, Before direct comparing cortical topography with human, we must be aware that in case of rodent, theta oscillation recorded by EEG is generated in hippocampus and volume-conducted, different from human EEG detected cortical theta oscillation. This difference originated from anatomy of brain in human and rodent. When it comes to amplitude changes following 40 hours of sleep deprivation in human, low frequency power (1-8Hz) increased during REM sleep in frontal dominant manner[41]. The increase power is similar to our topography in SR1 at frequency between 1 to 7Hz. However, in contrast to our result at 8-12Hz, which power is increased more than lower frequency in centro-medial region, human topography showed decreased power at 8-11Hz in global region.

2. Spectra changes after sleep deprivation

Power spectrum data showed that ranges of peak theta frequency during REM sleep became broaden through the days of sleep restriction as power prominently increased in 8 - 9Hz, which is higher theta than Peak theta frequency of baseline (7Hz). In the first day of sleep deprivation, change of power spectra was similar to total sleep deprivation study using rotating cylinder study in rat. The peak frequency was consistently 7 - Hz and increased power was peaks at 8 – 9 Hz after 24-h of sleep deprivation .

3. Theta peak frequency bifurcation in spectra

Furthermore, peak theta frequency showed bimodal distribution in repeated sleep deprivation. The reason why increased power of theta was beyond peak frequency was not unclear. Theta frequency recorded from rodent parietal cortex was originated from

hippocampal formation[39]. Modified Rhythmic activity of theta band in hippocampus after REM sleep deprivation was reported in rat. REM sleep deprivation consistently impaired spatial learning in Morris water maze test. The study showed that after learning process hippocampal theta rhythm was disturbed in REM sleep deprived rat. They also investigated alteration of the theta resonance of neuron and explain the modified theta activity as the ion current changes and therefore decreases in neuron excitability after REM sleep deprivation[44]. Because the spectra during REM sleep was not calculated, and deprivation protocol was different, It was hard to direct compare with our results. However, altered theta oscillation after REM sleep deprivation might be consistent in total sleep deprivation, reflected by changes of power spectra.

In this section, we are going to examine papers of two different type of study. One was the theta frequency changes as function of brain temperature, focusing in REM sleep. Another study was the report of theta peak frequency during wake and two types of REM sleep – tonic REM and phasic REM.

One study showed changes of power spectra during REM sleep. It was proposed that brain temperature could affect EEG along with circadian rhythms. The increased theta peak frequency during REM sleep previously reported as the function of brain temperature. EEG theta frequency is shifted significantly in the spontaneous brain temperature between 33 and 37‘c in vivo in the Djungarian hamster, nocturnal rodent. More specifically, increased cortical temperature from 34 to 36’c is related to more than 0.5Hz peak frequency shift during REM[45]. Brain temperature regularly changes during sleep and wake cycle, rising during the wake and REM sleep period, falling in NREM sleep. Consequently, sleep deprivation, which prolonged wake state, increased brain temperature. However, the increased brain temperature returned to baseline level during the first hour of recovery sleep[46]. In lines of the study, cortical temperature increased along the REM sleep duration was found[47], in other words, increased REM sleep duration reflected by peak theta frequency shift during REM sleep. Therefore, in series of studies, we guess that theta frequency was increased as the function of REM sleep duration because REM sleep duration and brain temperature has positive correlation. As

conforming the relationship between REM duration and theta frequency in our data, We found that weak positive correlation between the duration and theta peak frequency in our data was decreased across the sleep deprivation, comparing with baseline R value.

Another study showed that the peak theta frequency during wake and two types of REM sleep in B6 mice. Tonic REM has theta peak frequency in 6 – 7 Hz, phasic REM has in 9 – 10 Hz. Between the peak theta frequency of tonic REM and phasic REM, wake state has the peak theta frequency – about 8 – Hz. We could find that several individual spectra of a REM sleep episode clearly had two peaks. One peak was mostly in 7.5Hz and another peak was in 9.5Hz. Assuming that theta peak of B6 mouse is similar to our mouse – B6/129 hybrid F1, we guessed that some spectra showing 2 peaks was consist of both tonic REM and phasic REM, otherwise most spectrum had one peak in lower frequency, only representing tonic REM. Surprisingly clear double peak was found mostly in BL and recovery days, while average power spectra shows two peaks in SR5. In close looking, the higher peak in averaged spectra on SR5 was located about 9 – Hz, which is more like peak theta frequency during wake than phasic REM. Therefore, we supposed that another theta oscillator was emerged across the sleep deprivation, rather than increased amount of phasic REM (Fig.8).

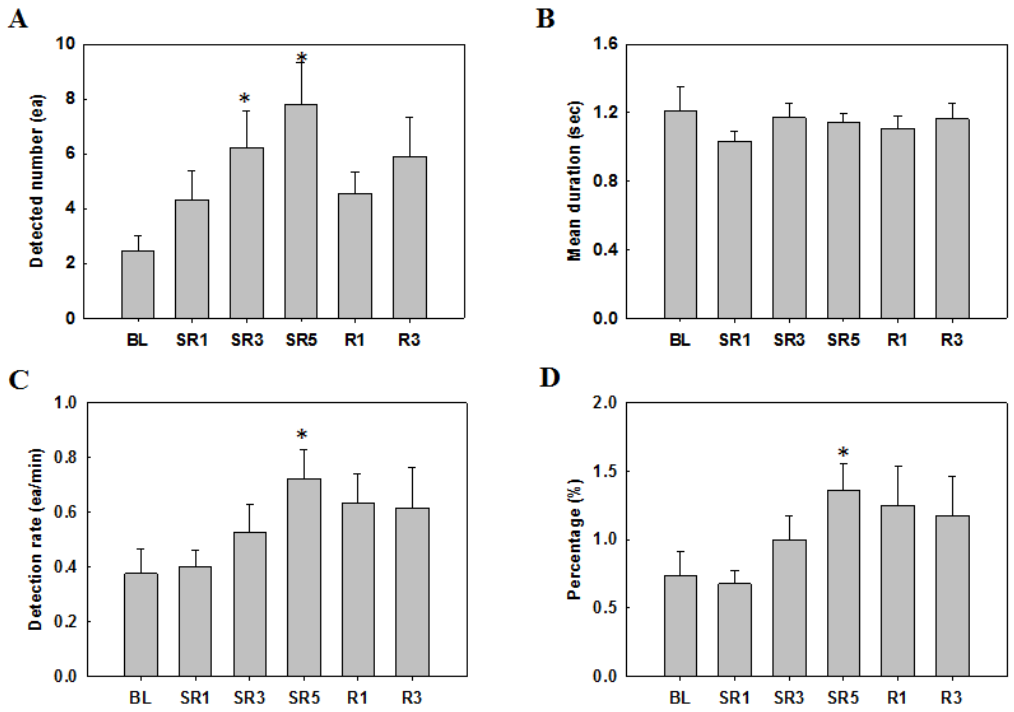

Figure 8. The detection of phasic REM changes during chronic sleep deprivation

(A) Detected number of phasic REM during 2 hours of sleep opportunity from ZT 1 to ZT 3 after 18 hours of sleep restriction on SR days, and corresponding time block on BL and R days (N=9) . The number of phasic REM gradually increased during CSR. (B) Mean duration of each episode of phasic REM conserved during CSR. (C, D) The rate of phasic REM detection and the percentage of total phasic REM time to total REM sleep time significantly increased on SR5. However, the amount of increase was not enough to affect averaged results.

The error bars indicate a standard error mean. Asterisks (*) indicate P-value lower than 0.05.

V. ITEMIZED SUMMARY

1. High density EEGs were acquired during natural, five successive sleep deprivation days, and three successive recovery sleep days in mice using a feather-shaped microarray.

2. Sleep deprivation reduces the total sleep time, but lengthens the REM sleep time.

3. In the first sleep deprivation day, the power in the theta band (5 ~ 10 Hz) increased significantly in global brain regions, whereas in the chronic sleep restriction days, the power in the low theta band (5 ~ 7.5 Hz) and one in the high theta band (7.5 ~ 10 Hz) behave oppositely. The low theta power decreased significantly, whereas the high theta power remained to be increased over the whole cortical area.

4. The chronic sleep restriction leads the gamma oscillations in the low frequency band (30 ~ 50 Hz) to increase in the frontal regions and the gamma oscillation in the high frequency band (65 ~ 100 Hz) to increase in the centro-parietal regions. The frontal low gamma increased monotonically during chronic sleep restriction.

5. A phase-amplitude relationship was observed between theta and high gamma oscillations in a region specific way. During baseline sleep, a nesting of gamma oscillation in high frequency band (65 ~ 100 Hz) by the phase of theta band (5 ~ 10 Hz) was observed in centro-parietal cortex. This modulation of gamma oscillation by theta phase was neither deteriorated nor enhanced by chronic sleep deprivation. On the other hand the modulation by theta phase was weakened in all the frequency band and in all the cortical regions except high gamma in centro-parietal cortex.

VI. CONCLUSION

In this thesis, I studied how REM sleep is affected by chronic sleep deprivation by utilizing recently developed high density EEG for mouse brain. Main findings of my study are that chronic sleep deprivation significantly influenced the sleep structure as well as EEG characteristics in REM sleep. First of all, the duration of REM sleep decreased as sleep deprivation continued, resulting in the ratio of REM sleep to total sleep time increased significantly in both acutely and chronically deprived sleep conditions. Secondly, chronic sleep deprivation altered the EEG characteristics during REM sleep and it was found that the changes of EEG rhythms were different between acute and chronic sleep deprived conditions. For example, the theta rhythm, a most conspicuous rhythm in REM sleep behaves in a different way such that a general increase in both frequency and region appeared in acute sleep deprivation, whereas a biphasic pattern, represented by the decrease of slow theta and increase of fast theta came along in chronic sleep deprivation. Furthermore, spectral analysis as well as bifurcation diagram of peak frequency evinced this switch of theta rhythm from unimodal to bimodal oscillation owing to chronic sleep deprivation. On the other hand, gamma oscillation changed as well depicted by an increase of slow gamma in the anterior frontal cortex and increase of fast gamma oscillation in centro-parietal cortex. I further analyzed the coupling between theta and gamma by calculating the cross-frequency coupling between theta phase and gamma power. Interestingly, the peak frequencies of theta and gamma for the maximal coupling value was preserved. However, the gamma coupling frequency band of theta was shifted to the higher frequency in chronic sleep deprivation. In sum, I found that chronic sleep deprivation regulates REM sleep in a significant level, implying that repeated sleep loss may severely subdue the benefit of REM sleep.

GLOSSARY

Electroencephalogram (EEG, 뇌전도) : The electroencephalogram (EEG) is a measure of brain waves. It is a readily available test that provides evidence of how the brain functions over time.

Delta waves（델타파）: Delta waves have a frequency of less than 3 cycles per second. These waves are normally found only when you are asleep or in young children.

Theta waves（세타파）: Theta waves have a frequency of 4 to 7 cycles per second. These waves are normally found only when you are asleep or in young children.

Gamma waves（감마파）: gamma waves have a frequency of 30 to 100 cycles per second. These waves are normally found during sleep and learning behavior.

NREM sleep : NREM (non-rapid eye movement) sleep is dreamless sleep. During NREM, the brain waves on the electroencephalographic (EEG) recording are typically slow and of high voltage, the breathing and heart rate are slow and regular, the blood pressure is low, and the sleeper is relatively still. NREM sleep is divided into 4 stages of increasing depth leading to REM sleep.

REM (R) sleep : Rapid eye movement sleep is deeper than non-REM sleep. During REM sleep, the eyes and eyelids flutter. Breathing becomes irregular. It is normal to have short episodes when breathing stops (apnea). You do most of your dreaming during REM sleep. But your brain paralyzes your muscles so you do not act out the dreams.

Homeostasis（항상성）**:** self-regulating process by which a system remains stable by adjusting to changing conditions (Dendritic Spines Lab, Virtual Neurons)

Circadian（일주기）**:** Refers to events occurring within the span of a full 24-hour day, as in a circadian clock.

Hippocampus : the oldest part of cerebral cortex responsible for spatial localization, formation of declarative memory, and transfer of short-term to long-term memories (Sheep Brain Dissection, Memory Items)

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Abstract (in Korean)

만성적 수면박탈 마우스에서 나타나는 REM 수면 뇌파의 변화

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만성수면박탈은 현대사회에서 빈번하게 일어나는 현상으로, 반복적으로 박탈된 수면량의 누적은 수면시간외 피곤함 호소, 집중력과 인지기능을 저하시키고 잠정적으로 심장질환의 발병 위험도를 증가 시키며, 사고 발생률을 높이게 된다는 보고가 있다.

수면은 일주기와 깨어있던 시간에 비례하여 조절된다. 깨어있는 시간이 늘어나면 수면 필요량이 증가하고 항상성 기작으로 수면시간의 증가와 NREM 수면에서 기록한 뇌전도의 느린 파형의 진폭이 증가한다. 그러나 반복된 수면 박탈의 경우 수면의 항상성이 파괴되고 증가된 느린 파형의 진폭이 오히려 정상 상태로 돌아오는 현상도 알려진 바 있다.

REM 수면에서는 단기 수면박탈로는 그 변화가 일정하지 않으나, 장기간 반복된 수면 박탈의 경우 랫에서 NREM 수면 보다 더 정확한 비율로 그 시간이 증가한다는 보고가 있다. 그러나 만성적인 수면 제한 실험에서는 REM 수면의 변화를 집중적으로 다룬 결과는 보고되지 않고 있다. 때문에 본 연구에서는 유전자 조작 마우스의 정상 대조군으로 쓰이는 B6/129 에서 기존의 랫 모델과 같은 방식의 만성 수면박탈을 진행하면서 뇌파의 변화를 기록하고, 특히 REM 수면 상태의 뇌파변화에 집중하였다.

9 마리의 마우스가 실험에 사용되었으며, 실험 일정은 첫날의 기저 수면량에 대한 기록을 포함하여, 5 일동안의 수면박탈 후 3 일동안 회복기까지 총 9 일에 걸쳐 진행되었다. 수면박탈은 하루에 총 18 시간동안 진행하였고, 이후 6 시간 동안은 마우스를 우리에 옮기고 잠을 잘 수 있는 휴지기를 주었다. 수면가능시간의 첫 1 시간 이후부터 2 시간 동안, 고해상도 박막전극을 이용하여 뇌전도를 기록하고 분석하였다.

수면이 가능한 6 시간 동안 REM 수면의 양은 기저수면상태에 비해 수면박탈 이후에 증가를 보였다. 특히 총 수면 양이 줄어듦에도 REM 수면 양은 감소하지 않기 때문에, 총 수면량 중 REM 수면이 차지하는 비율은 수면박탈기간이 지속됨에 따라 선형으로 증가하였다.

뇌파 진폭의 변화를 보기 위해 시행한 스펙트럼 분석에서는 낮은 세타파의 영역(5 - 7 Hz)의 진폭이 수면박탈의 첫날에 유의미하게 증가하였으나 수면박탈이 더 진행됨에 따라 셋째 날과 다섯째 날에서는 오히려 기저상태보다 감소함을 보였다. 반면, 높은 세타파 영역 (7 - 10 Hz)는 수면박탈 기간 동안 지속적인 증가를 보였으며, 이러한 증가는 특히 중심 두정부위에서 특히 두드러졌다. 스펙트럼 분석을 평균하여 관찰한 결과 이러한 변화는 낮은 세타파의 영역인 7 - Hz 에서 최고점을 보이던 기저상태에 비해, 수면박탈이 진행에 따라 7 - Hz 와 9 - Hz 에서 두 개의 최고점을 가지는 쌍봉형태의 스펙트럼으로 변화하기 때문인 것으로 보여진다.

세타영역 외에도 REM 수면동안 감마파 영역의 리듬의 증가가 알려져 있으므로 낮은 감마영역 (30 - 50 Hz)과 높은 감마영역 (70 - 100 Hz)의 증감 또한 분석에 포함하였다. 이 두 종류의 감마 영역은 모두 수면박탈이 진행됨에 따라 증가되는 진폭을 보였다. 그러나 낮은 감마영역의 경우 전두엽에서 뚜렷한 증가를 보이는 반면, 높은 감마영역의 진폭 증가는 주로 중심-두정부위에서 가장 뚜렷하였다.

마지막으로 깨어있는 상태에서 기억학습을 반영하는 것으로 알려져 있는 세타파에 의한 감마파의 조절 현상을 알아보기 위하여, Cross-Frequency-Coupling (CFC) 분석을 수행하여, 수면박탈에 따른 세타파와 감마파의 조절량 변화를 보고자 하였다. CFC 의 결과는 가장 큰 조절량을 나타내는 8Hz 와 70Hz 의 CFC 강도는 만성수면박탈 중 변화하는 뇌파의 진폭 변화에도 불구하고 일정하다는 것을 보여준다.

핵심 되는 말 : 만성수면박탈, REM, 감마파, 세타파, CFC

PUBLICATION LIST

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